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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 09/897,988
Filing Date: July 05, 2001
Appellant(s): NAKAI ET AL.

Shelly Cermak
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed 1/5/11 appealing from the Office action mailed 10/13/10.

(1) Real Party in Interest

The examiner has no comment on the statement, or lack of statement, identifying by name the real party in interest in the brief.

(1) Real Party in Interest

The examiner has no comment on the statement, or lack of statement, identifying by name the real party in interest in the brief.

(2) Related Appeals and Interferences

The following are the related appeals, interferences, and judicial proceedings known to the examiner which may be related to, directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal:

102 rejections of claims in this application were previously appealed to the Board of Patent Appeals and Interferences, Appeal No. 2009-4917, which were reversed in a Decision dated August 11, 2009.

(3) Status of Claims

The following is a list of claims that are rejected and pending in the application:

Claims 1, 6 and 12-17 are pending and rejected.

The examiner has no comment on the appellant's statement of the status of amendments after final rejection contained in the brief.

(5) Summary of Claimed Subject Matter

The examiner has no comment on the summary of claimed subject matter contained in the brief.

(6) Grounds of Rejection to be Reviewed on Appeal

The examiner has no comment on the appellant's statement of the grounds of rejection to be reviewed on appeal. Every ground of rejection set forth in the Office action from which the appeal is taken (as modified by any advisory actions) is being maintained by the examiner except for the grounds of rejection (if any) listed under the subheading "WITHDRAWN REJECTIONS." New grounds of rejection (if any) are provided under the subheading "NEW GROUNDS OF REJECTION."

(7) Claims Appendix

The examiner has no comment on the copy of the appealed claims contained in the Appendix to the appellant's brief.

(8) Evidence Relied Upon

5830716	Kojima et al	11-1998

Kusomoto et al, Menaquinol oxidase activity and primary structure of cytochrome bd from the amino-acid fermenting bacterium Corynebacterium glutamicum,, Arch Microbiol (2000) 173:390-397

Calhoun et al, Energetic efficiency of Escherichia coli: effects of mutations in components of the aerobic respiratory chain, J Bacteriol. 1993 May; 175(10): 3020-3025

Sone et al, Collection of Summaries of Lectures made at the Meeting of Japan Bioengineering Association, September 15, 1995, p. 10

DT Ciccognani et al., FEMS Microbiology Letters,"Carbon monoxide-binding properties of the cytochrome bo quinol oxidase complex in Escherichia coil are changed by copper deficiency in continuous culture," 1992, 94, 1-6

(9) Grounds of Rejection

Claims 1, 6 and 12-17 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kojima et al (US 5,830,716; see entire document) in view of Calhoun et al (J Bacteriol. 1993 May; 175(10): 3020–3025; see entire document), Ciccognani et al (FEMS Microbiology Letters 94, 1992, page 1-6; see entire document) or Kusomoto et al (Arch Microbiol, 2000, Vol 173, pages 390-397; see entire document) or Sone et al (Collection of Summaries of Lectures made at the Meeting of Japan Bioengineering Association, September 15, 1995, p.10).

Kojima et al provide methods of using bacteria for production of amino acids. Cells are grown in culture wherein the target substance is produced in the culture medium and isolated thereof (see e.g. abstract). Specifically, Kojima et al teach that E. coli and Coryneform bacteria are well known in production methods of threonine, lysine and phenylalanine wherein the cells

are engineered to improve production by altering a biochemical cellular pathway (see e.g. col 3. line 55-col 4, line 35).

Kojima et al description of altered biochemical pathways does not include one wherein the high energy efficiency pathways (nuo and cytochrome bo) and low efficiency pathway (ndh and cytochrome bd) are altered.

However, **Kusomoto** et al teaches that cells used for production methods of amino acids can be altered for improved amino acid production by altering the aerobic metabolism of the cell. Specifically, by deleting the low efficiency gene.

> "In order to improve the efficiency of cell growth and amino acid production, it is important to understand the aerobic energy metabolism or, more specifically, the respiratory proton pumps in the bacterium."
>
> "Cytochrome bd-type oxidase has been shown to have a lower H^+/O ratio than haem-copper oxidases (Miller and Gennis 1985; Puustinen et al. 1991). It has been reported that the H^+/e^- ratio is about 1 for intact cells of C. glutamicum with endogenous substrate. This is lower than that expected if an aa_3-type haem-copper oxidase is operating (Kawahara et al. 1988). Thus, it is likely that deletion of the cytochrome bd genes would increase the H^+/e^- ratio of the respiratory chain, the efficiency of energy metabolism, and consequently the growth yield of the bacterium."

Hence, Kusomoto et al directly link increased amino acid production with the growth yield of the cell and the energy efficiency of the cell.

It is established in the art that the energy efficient pathways of a number of microorganisms comprise high and low efficiency pathways and that alteration of the pathways can increase the energy efficiency of the cells and alter the growth yield. High energy efficiency pathways include nuo which encodes NHD-I and cytochrome bo and low efficiency are ndh which encodes NDH-II and cytochrome bd.

For example, **Calhoun** et al teach

> "In principle, by directing the electron flux through specific respiratory components, the energetic efficiency of the E. coli respiratory chain can be varied between 4H+/e- (with NDH-1 and the bo-type oxidase) and 1H+/e- (with NDH-2 and the bd-type oxidase). Since the wild-type strain contains both NADH dehydrogenases and both terminal oxidases, the value for the H+/e- ratio must fall between these two extremes and will vary with growth conditions."

Calhoun et al assesses growth efficiency by constructing strains to use only one NADH dehydrogenase and one terminal oxidase and determines,

> "the data confirm the following expectations based on the in vitro proton translocation measurements: (i)based on the in vitro proton translocation measurements: (i)the elimination of the uncoupled NDH-2 results in increased energetic efficiency; (ii) strains that utilize the bd-type oxidase have a less-efficient respiratory chain than those using the bo-type oxidase."

Hence, Calhoun explicitly teaches that to increase growth efficiency, one would eliminate NDH-II or bd and increase bo. Results from nuo are not demonstrated because at the time of publication it had not been cloned. This reference nonetheless directs one to create the strains recited in claims 1, 6, 7 and 11.

Specifically, Calhoun et al explicitly and inherently teaches strains in which bo is increased, ndh II is decreased by gene disruption or both bo is increased and ndhII is deficient. Explicitly, Calhoun teaches that strains with deleted ndh-II has been created and produces cells with increased growth yield. (These strains are encompassed by Sone et al who teaches that strains comprising bo cytochrome oxidase activity and that lack bd cytochrome oxidase activity has enhanced growth yield in E. coli (see Results).

By directing one to create cells with increased bo, Calhoun et al inherently directs one to increase copy number of bo. For example,

Ciccognani et al teach methods of culturing E. coli (RG145), which is a genetic recombinant strain in which an enzyme of the high-energy efficiency pathway was enhanced and

an enzyme of low-energy efficiency was deficient. The cells contain a chromosomal deletion resulting in the inability of the cell to express cydA and contain a cosmid containing the cyo operon resulting in over expression of the cytochrome bo complex (page 2, section 3.1).

In KSR International Co. v. Teleflex lnc., 82 USPQ2d 1385 (U.S. 2007), the Supreme Court particularly emphasized "the need for caution in granting a patent based on a combination of elements found in the prior art," (Id. At 1395) and discussed circumstances in which a patent might be determined to be obvious. Importantly, the Supreme Court reaffirmed principles based on its precedent that obviousness in part is predicated on use of particular known techniques that are recognized as part of the ordinary capabilities of one skilled in the art.

In the instant case, it is accepted that production methods of amino acids utilize E. coli and coryneform bacteria in which the cells are cultured and the produced amino acids are excreted and isolated from the cell culture and that these methods can be improved with predictable results by applying known techniques of cellular engineering for improved energy efficiency.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to use microorganisms that have been altered to have enhanced high efficiency pathways and/or deficient low energy pathways given that these modifications are taught by multiple sources such as Calhoun et al, Ciccognani et al, Sone et al or Kusomoto et al with the known methods of producing amino acids using the methods reviewed by Kojima et al because Kojima et al teach that it is within the ordinary skill of the art to use E. coli to produce amino acids wherein the method requires culturing of and isolation from the culture of amino acids and because Calhoun et al, Ciccognani et al, Kusomoto et al, and Sone et al teach that production

cells can be improved by altering the energy efficiency pathways of the cells and more specifically Kusomoto et al teach that the increase in energy efficiency as well as in improved growth yield is attributed to an improvement in the amino acid production in such strains. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

(10) Response to Argument

First, applicants argue that the rejection is in error. Starting on page 12, applicants argue that neither Calhoun, Ciccognani et al or Sone et al describe the effect of alterations in the energy efficiency on L-amino acid production. As well, applicants argue that a person of skill in the art would know that when increasing growth yield one would expect L-amino acid production to decrease. As well, applicants point out that neither Calhoun et al nor Kusimoto et al teach an increase in cytochrome bo nor cytochrome bd altered.

Applicants' arguments filed have been considered but are not persuasive for the following reasons. Kojima et al based upon Kusomoto et al must be considered as a whole and combined the references direct a skilled artisan to alter an E. coli or Corneyform cell to improve amino acid production in these cells. Specifically, Kojima teaches that E. coli and Corneyform cells are known in the art to be amino acid producers, and directs one to improve upon their design. Kusomoto et al teach that amino acid production is improved by altering the energy efficiency which further results in improved cell yield. Hence, a person of skill in the art would have at the time of filing identified means to improve E. coli or Corneyform cells and would

have identified as one of these methods to improve the energy efficiency of the cell wherein the alteration would result in improved cell growth as well as in amino acid production. Specifically Kusomoto et al, directly link alteration of the energy efficiency of the cell with metabolism (amino acid production) and growth yield. And more specifically, Kusomoto et al teaches deletion of cytochrome bd (emphasis added).

> "Corneybacterium glutamicum is an aerobic, gram-positive high-G+C bacterium that is of industrial importance in producing amino acids used as nutritious additives to food and feed. In order to improve the efficiency of cell growth and amino acid production, it is important to understand the aerobic energy metabolism or, more specifically, the respiratory proton pumps in the bacterium."
>
> "Cytochrome bd-type oxidase has been shown to have a lower H^+/O ratio than haem-copper oxidases (Miller and Gennis 1985; Puustinen et al. 1991). It has been reported that the H^+/e^- ratio is about 1 for intact cells of C. glutamicum with endogenous substrate. This is lower than that expected if an aa_3-type haem-copper oxidase is operating (Kawahara et al. 1988). Thus, it is likely that deletion of the cytochrome bd genes would increase the H^+/e^- ratio of the respiratory chain, the efficiency of energy metabolism, and consequently the growth yield of the bacterium."

To this end, the teachings of Calhoun et al, Ciccognani and Sone et al satisfy a need left by the teachings of Kojima et al in view of Kusomoto et al. Specifically, the teachings therein direct one to find ways in which the energy efficiency of a cell can be improved as the determination of Kojima et al in view of Kusomoto et al are that L-amino acid production is improved by engineering in which the cell has increased growth yield and energy efficiency thus leading t o improved amino acid products. More specifically, it is known that to alter the efficiency of energy of a cell that four types of enzyme activity must be altered. Cytochrome bd and NDH-II operate on a low energy efficiency while cytochrome bo and NDH-I operate as high energy efficiency enzymes (see introduction and figure 1 of Calhoun et al).

Ciccognani et al is directed specifically to increasing copy number of cytochrome bo oxidase and therefore Calhoun and Kusomoto et al need not. Rather, Calhoun et al investigates the effect of deletion of cytochrome bd, cytochrome bo and a combination of cytochrome bo + ndhII, he also demonstrates the importance of maintaining bo type oxidase which demonstrates

enhanced bioenergetics efficiency. As well, deletion of ndh-II also resulted in increased energy efficiency, presumably by increasing reliance on ndh-I. The strains of Ciccognani et al. have elevated cytochrome bo and lack cytochrome bd. That the strains therein are regulated by cooper does not detract from the fact that the strains have altered energy efficiency and are well known in the art. The strains of Sone et al are a recapitulation of the strains of Calhoun et al. Applicants argue that the reference is based upon the conclusion of the Japanese Examiner which reviewed Sone et al. However, this reference is provide as it represents what a person of skill in the art would conclude and that is that that these modifications improve metabolism, amino acid production by altering the growth yield and energy efficiency of the cell. To this end, all of the instant steps are available in the art and applicable together under the principles of KSR.

> If a person of ordinary skill can implement a predictable variation, § 103 likely bars its patentability. For the same reason, if a technique has been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond his or her skill. Id. at ,82 USPQ2d at 1396.
> When considering obviousness of a combination of known elements, the operative question is thus "whether the improvement is more than the predictable use of prior art elements according to their established functions." Id. at, 82 USPQ2d at 1396.

In this case, Kojima teaches that methods of producing and collecting L-amino acids from the cell are routine in the art and direct one to engineer cells in order to improve such methods. As well, the art teaches that improved conditions include those that improve high energy efficiency pathways in the cell which the art also demonstrates includes a number of such alterations which are also routine in the art. In other words, the principle of improving growth yield by improving energy efficiency of the cell has been established and the steps of exploiting such a cell to improve amino acid production as directed by Kojima and Kusomoto et al does not appear to be

an inventive step. Rather applying known techniques of engineering cells for improved bo expression and decreased ndhII to known techniques is an interchangeable product wherein an improvement in the method of producing L-amino acid collection would occur without reasonable doubt.

Secondly, applicants provide arguments and evidence that improved growth yield does not lead to improved production of L-amino acids. #1 Eggeling et al is directed to methods of altering dapA enzyme in the biosynthetic pathway that exists at the branch between L-lysine and L-threonine production. Hollander et al teach that the limitation of phosphorous/ carbon is directly responsible for reduced biomass (see e.g. col 3, line). In response, it is noted that the teachings of Hollander et al and Eggeling et al are not commensurate in scope with the instant claims and because the recombinant mehcanisms differ, the metabolic effects on the cell cannot be compared. Specifically, the instant rejection directs one to improve biosynthetic pathways to improve L-amino acid production (Kojima et al) by altering energy efficiecny (Kusomot et al) which will concommitantly increase growth yield.

> it is likely that deletion of the cytochrome bd genes would increase the H_+/e_- ratio of the respiratory chain, the efficiency of energy metabolism, and consequently the growth yield of the bacterium."

The alterations and effect of biomass by alterations of unrelated biosynthetic pathways does not provide the proper basis to argue against the above observation. In the case, of Eggeling et al, the limits on growth are a consequence of the recombinant techniques utilized to overexpress dapA. To this Eggeling et al teach,

> "A global response of the carbon metabolism to the synthase activity becamse apparent:the increased flux towards lysine was accompanied by a decreased flux towards threonine. This reusled in a decreased growth rate, but increased intracellular levels of pyruate-derived valine and alanine. Therefore,

> modulating thelfux at the branc point results in an intrinsically introduced growth limtiation with increased intracellular precursor supply for lysine synthesis".

Specifically, this teaches a specific metabolic alteration that by reducing threonine yield reduces growth yield but due to the flux in the metabolic pathway drives lysine production. To the contrary, the instant claims teach a global alteration in energy efficiency with the direct consequence of improved energy efficiency that leads to increased amino acid production. In noe way, can Eggeling et al be taken to argue that any global reduction in growth yield will lead to improvde amino acid production. It is inherent in the atlerations produced by Eggeling et al that the growth yield is concommitant with increased lysine and that is trhough reduced threonine. The cell is responsive to the decreased flux towards threonine by reducing growth yield and lysine production.

Similarly, Hollander et al is not directed to the effect of genetic alterations on amino acid yeild. In the case of Hollander et al the limitation on biomass is a consequence of limiting carbon and/or phosphorous which step is not utilized in the instant rejection. Rather, the genetic alterations of the instant claims are designed to modulate the cell for improved growth yield and improved amino acid production wherein phoshorus and carbon are not limited. The genetic modificatiosn are desinged to improve the yield of the lysine in a manner that obviates the concerns over biomass and needs to grow cultures greater than 400 hours, which concern palgues the non genetically altered strains of Hollander et al. And in support of such methods, Kusimoto et al demonstrate that concommitant with improved yield, imporved amino acid production is boatined by alterations in the energy efficiency of the cell.

Therefore, the method of recombinant engineering of Eggeling et al and the nutrient limitation of Hollander et al differ significantly from the methods of Kojima et al in view of Kusomoto et al wherein ancillary effects in the growth rate would not necessarily be the same for both. Rather, the instant rejection improves energy yield by recombinant methods of altering energy pathways, a technique that is unrelated to the methods of Hollander et al or Eggeling et al. In so doing, the energy yield of the cell is improved, amino acid production is improved and growth yield is improved. To this end, the MPEP 2145 teaches, "In re Huang, 100 F.3d 135, 139-40, 40 USPQ2d 1685, 1689 (Fed. Cir. 1996). See also GPAC, 57 F.3d at 1580, 35 USPQ2d at 1121; In re Paulsen, 30 F.3d 1475, 1482, 31 USPQ2d 1671, 1676 (Fed. Cir. 1994) (Evidence of commercial success of articles not covered by the claims subject to the 35 U.S.C. 103 rejection was not probative of nonobviousness.). Additionally, the evidence must be reasonably commensurate in scope with the claimed invention. See, e.g., In re Kulling, 897 F.2d 1147, 1149, 14 USPQ2d 1056, 1058 (Fed. Cir. 1990); In re Grasselli, 713 F.2d 731, 743, 218 USPQ 769, 777 (Fed. Cir. 1983). In re Soni, 54 F.3d 746, 34 USPQ2d 1684 (Fed. Cir. 1995) does not change this analysis. In Soni, the Court declined to consider the Office's argument that the evidence of nonobviousness was not commensurate in scope with the claim because it had not been raised by the examiner (54 F.3d at 751, 34 USPQ2d at 1688)."

It is noted that applicants appear to be arguing that the references should be analyzed in independently in combination with Kujima. However, based upon previous arguments, it is not clear how this interpretation has prevailed. The Final Rejection has stated, "In this case, applicants have attacked Kojima for lack of discussion of high energy and low energy efficiency pathways, Calhoun for not teaching that increasing cell growth correlates with increased amino

acid production, Sone, Spehr and Ciccognani as being limited to analysis based upon alteration of high/low energy efficiency pathways that are not related to L-amino acid production and Kusimoto for lack of teaching the relationship of bo enzyme to L-amino acid production. Rather, the obviousness of a method of producing an L-amino acid by culturing a bacterium capable of accumulating the L-amino acid in the medium and furthermore comprising enhanced bo-type oxidase activity is based upon the skill of one in the art and the predictability of the combination of events. Specifically, the combination of references established that it is routine to use bacterium capable of producing L-amino acids for collection from the medium and furthermore that these bacterium can be "engineered" for improved production." To which it is clear applicants understood that the references were to be combined. From the arguments 9/13/10, " Despite applicant's sound arguments presented previously, the Examiner maintains the rejection by combining Kojima, which allegedly discloses L-amino acid fermentation, with Calhoun, Ciccognani, Kusomoto, and Sone, which allegedly suggest NDH-2 disruption, cyo operon-amplification, cytochrome bd oxidase disruption and cytochrome bd oxidase amplification lead to improved growth yield of bacteria. However, as previously argued, improved growth yield does not lead to an improvement in the production of L-amino acids.".

Furthermore, the rejection clearly sets forth the basis of the rejection as based upon a review of the art and through such review a clear establishment that the art as a whole teaches 1) that E. coli and Coryneform bacteria are well known in production methods of threonine, lysine and phenylalanine wherein the cells are engineered to improve production by altering a biochemical cellular pathway (Kujima et al) 2) wherein the art has established a direct link between increased amino acid production with the growth yield of the cell and the energy

efficiency of the cell (Kusomoto et al). To this end, the art is replete with teachings that establish means of altering the energy efficiency in the cell wherein 3) for increased growth efficiency, one would eliminate NDH-II or bd and increase bo (Calhoun and Sone et al) or 4) strains such as have been altered to comprise an enzyme of the high-energy efficiency pathway enhanced and an enzyme of low-energy efficiency deficient (Ciccognani et al). More specifically, the rejection states, "the known methods of producing amino acids using the methods reviewed by Kojima et al because Kojima et al teach that it is within the ordinary skill of the art to use E. coli to produce amino acids wherein the method requires culturing of and isolation from the culture of amino acids and because Calhoun et al, Ciccognani et al, Kusomoto et al, and Sone et al teach that production cells can be improved by altering the energy efficiency pathways of the cells and more specifically Kusomoto et al teach that the increase in energy efficiency as well as in improved growth yield is attributed to an improvement in the amino acid production in such strains". Hence, the reference sets forth that in the art it was well known to use E. coli and Coryneform bacteria to produce amino acids for isolation from the media wherein these bacteria are preferable altered to increase yield. A person of skill in the art looking for means to improve the cell lines to this end would have followed the analogous art teachings of Kusomoto et al that teach that alterations in the energy efficiency are desirable to improve amino acid yield and growth yield. Other alterations in the energy efficiency systems are presented that are known and developed in the art.

(11) Related Proceeding(s) Appendix

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

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Primary Examiner, Art Unit 1633

Conferees:

/Joseph T. Woitach/

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